BREEDING HABITS AND ASSOCIATED PHENOMENA IN SOME INDIAN BATS – PART XIII – MALE REPRODUCTIVE PATTERNS IN THREE BATS[1]

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(With a plate and four text-figures)

Male reproductive rhythm is reported in three species of Indian bats, namely *Rousettus leschenaulti, Hipposideros fulvus fulvus* and *Pipistrellus ceylonicus chrysothrix* from Aurangabad by examining the testes and accessory glands throughout the year. Males of *R. leschenaulti* have a long sexual season extending from October to the end of March, when they retain their copulatory competence. Males of *H. fulvus fulvus* come to sexual activity in a sharply defined period during November. Males of *P.c. chrysothrix* are sexually active from the first week of June to the second week of July. While males of *P.c. chrysothrix* attain sexual maturity within the year of birth, males of the other two species reach sexual maturity in 14 to 19 months. External factors do not seem to play a significant role in the onset of breeding activity.

Introduction

Most reports on the breeding behaviour of Indian bats are based on the examination of females (see Gopalakrishna and Sapkal 1986 for detailed bibliography) with a view to identifying the breeding season(s) on the basis of pregnancy record. Studies on the sexual rhythm of males have not been made except in two Indian bats, namely *Scotophilus temmincki (S. wroughtoni)* (Gopalakrishna 1948, 1949) and *Rhinopoma microphyllum kinneari* (*R. kinneari*) (Kumar 1965). In both these species the sexual rhythm is synchronous in the two sexes and hence, pregnancy record can be taken as the criterion for determining sexual periodicity. However, there are many species in which this can be deceptive because of considerable time lag between copulation and ovulation. In such cases the study of the sexual rhythm in males becomes essential for determining reproductive behaviour. We chose to study the males of *Rousettus leschenaulti* (Pteropodidae), *Hipposideros fulvus fulvus* (Hipposideridae) and *Pipistrellus ceylonicus chrysothrix* (Vespertilionidae) as they represent widely different families, exhibit different patterns of reproductive activity and bear different relationships to the sexual rhythm of the females. All the species were examined from the same geographical region.

No attempt is made here to describe the anatomy of the male genitalia of these species since this has already been reported (Gopalakrishna and Murthy 1976, Murthy 1971).

Material and Methods

The specimens of the three species were collected at and around Aurangabad (19° 53′ N, 75° 25′ E) in Marathwada region of Maharashtra. *H. f. fulvus* and *P. c. chrysothrix* are house bats inhabiting dark humid recesses of old houses, grain godowns and cow-sheds. A few specimens of *H. f. fulvus* and *P. c. chrysothrix* were collected at Nanded (19° 9′ N, 77° 20′ E) about 130 km south-east of Aurangabad and the condition of the genitalia of these specimens was similar to that of the Aurangabad specimens. A large colony of *R. leschenaulti* occurred in an underground tunnel near Bibi-ka-Mukbara in Aurangabad.

Specimens of all the species were collected randomly during 1964-1966 and 1976-1979, with at least one collection every calendar month (Table 1). The body weight of every

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specimen was recorded after killing by chloroform and the male genital organs were dissected out and immersed in alcoholic Bouin's fixative. After 24 hours of fixation the tissues were transferred to 70% ethanol. The right testis of each specimen was weighed in a Mettler balance after rolling the testis a few times on a filter paper to remove the excess fluid on the surface of the testis. Paraffin-embedded testes and accessory glands of selected specimens from each month's collection were sectioned at 10μ thickness. The section were stained with Ehrlich's haematoxylin and counterstained with eosin after following the usual procedure and mounted in DPX.

Interstitial cell counts were made following the method adopted by Gopalakrishna (1949).

Observations

Rousettus leschenaulti

This species breeds twice a year in quick succession (Gopalakrishna 1964, Gopalakrishna and Choudhari 1977). The first cycle commences in November-December and deliveries in the colony occur during March-April. The second cycle commences within a few days after parturition and deliveries occur during the latter half of July. Each female delivers a single young one during each cycle. The lactation period of the first cycle overlaps the early gestation period of the second. Females are sexually quiescent from August to the first week of November.

The newly born young male weighs 12 ±1 g. During the suckling period, which lasts for 35 to 40 days, the young one grows rapidly and attains a weight nearly three times its weight at birth. All specimens weighing more than 73 g are sexually mature and the specimens attain this weight at the age of 14-15 months (Gopalakrishna and Choudhari 1977). All specimens having a testis weight of 100 mg and over were sexually mature. The testis weight of adult animals does not fall below this even during the sexually quiescent period. Hence, apart from body weight, the weight of the testis can also be taken as a valid criterion for determining sexual maturity in this species.

Fig. 1 gives the relative increase in body weight and testis weight. This reveals some interesting features. The weight of the testis of the newly born young is 12 mg. By the time the body weight reaches 60 g (nearly five times the weight at birth) the weight of the testis reaches 20 mg — a little over 1.5 times the weight at

Table 1

MONTHWISE COLLECTION OF MALE SPECIMENS OF THREE SPECIES OF BATS

Month	*Rousettus leschenaulti*			*Hipposideros fulvus*			*Pipistrellus ceylonicus*		
	Immature	Adult	Total	Immature	Adult	Total	Immature	Adult	Total
Jan.	12	61	73 (6)	1	15	16 (7)	4	20	24 (9)
Feb.	19	43	62 (4)	—	5	5 (4)	—	10	10 (5)
March	7*+ 18	43	68 (6)	1	5	6 (6)	—	23	23 (7)
Apr.	38* + 19	52	109 (9)	4	4	8 (3)	—	20	20 (5)
May	3* + 13	30	46 (7)	15 + 20	16	51 (6)	—	36	36 (12)
June	23	15	38 (5)	5	18	23 (4)	—	42	42 (14)
July	2* + 8	9	19 (3)	1	9	10 (2)	—	56	56 (16)
Aug.	1* + 3	13	17 (3)	1	11	12 (4)	4*	25	29 (15)
Sept.	7	22	29 (5)	—	10	10 (4)	62*+ 11	12	85 (20)
Oct.	1	8	9 (2)	15	17	32 (5)	2* + 21	19	42 (10)
Nov.	23	45	68 (8)	—	19	19 (5)	9	22	31 (9)
Dec.	24	46	70 (6)	4	8	12 (6)	6	16	22 (7)
Total	51* + 170	387	608	19* +48	137	204	68* + 51	301	420

*Indicates number of sucklings in the month. Numbers in parentheses indicate the number of collections made during the respective month.

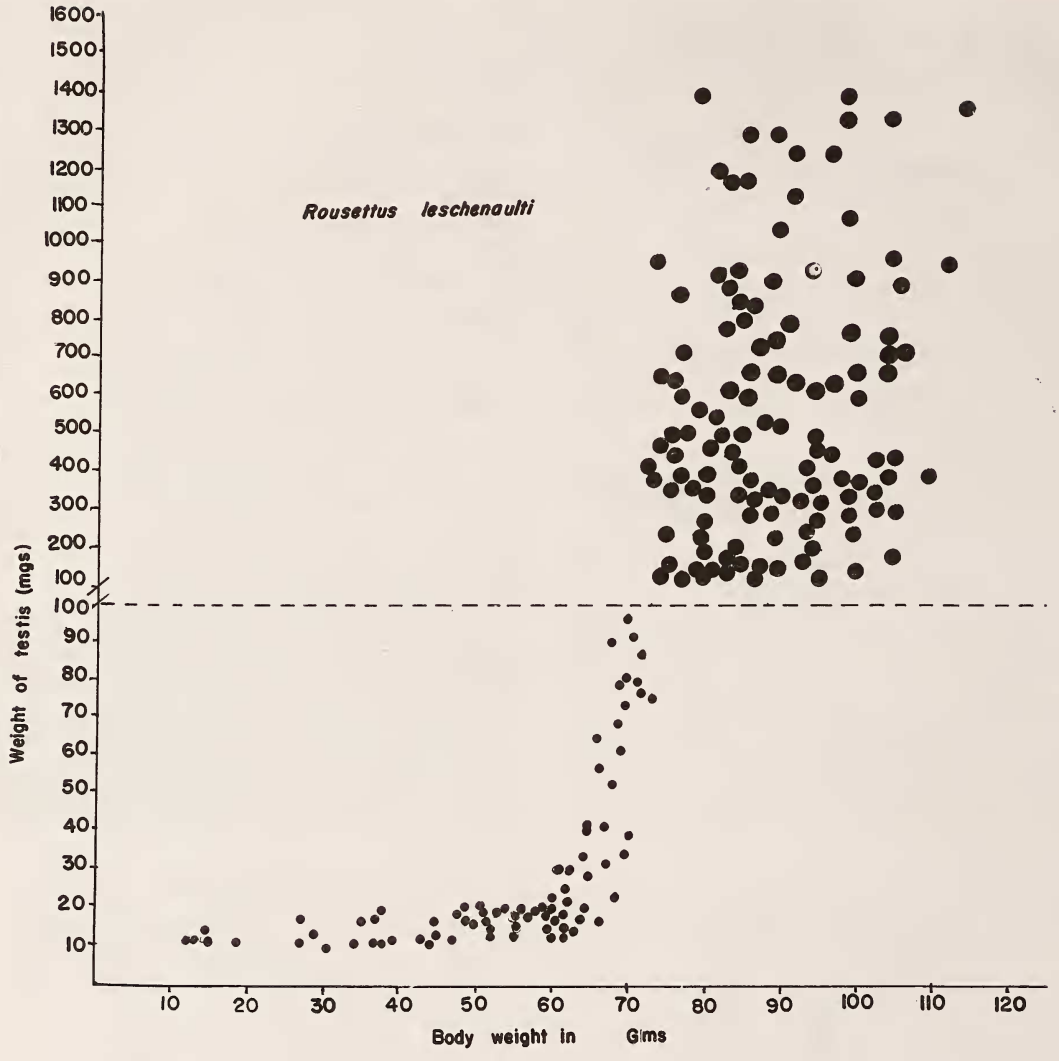


Fig. 1. Scatter diagram showing relationship between increase in body weight and weight of testes in *Rousettus leschenaulti*. The discontinuous line denotes weight at sexual maturity. See text for descriptions.

birth. After this stage until sexual maturity, the increase in body weight is only 13 mg (about 20%), whereas the testis attains a weight of 100 mg (an increase of nearly 500%).

During the sexually active season the colony contains a few immature bats (Plate 1, Figs. 5-9). This indicates that the animals do not attain sexual maturity within the year of their birth. During November to February the animals could be recognised as two distinct groups on the basis of testis size (Plate 1, Figs. 5-7), thereby indicating that the juveniles were nearly of the same age — and were most probably born during the previous breeding season. It was, however, not possible to distinguish the animals born in February-March from those born the

previous July. But from March onwards (Plate 1, Figs. 8, 9) three distinct groups of animals could be identified on the basis of testis size — those born in February-March of the year, those born during February-March and July of the previous year and adult animals, which were at least 20-24 months old.

Fig. 2 shows the weight of the testis of adult animals during the different months of the year. Testis weight is low during April to September, then increases suddenly during October-November. It falls a little during December-January, but not to the level as during April to September. There is a second rise during February and March, but this is not to the same peak as during November-December.

The testis does not exhibit any spermatogenetic activity from April to September. During October and November the seminiferous tubules become wider and there is a sudden spurt of spermatogenetic activity, and the lumen of the seminiferous tubules contains spermatozoa in large numbers. During December and January, although spermatogenetic activity continued, it was considerably less vigorous. A second spurt of spermatogenesis occurs during February and March, after which there is complete cessation of spermatogenesis until October.

Fig. 2 also includes a scatter diagram giving the total number of interstitial cells in the adult testis during different months of the year and the curve illustrates the variations in the number of these cells during the year. The curve has two peaks of activity closely parallelling those of the changes in the weight and spermatogenetic activity in the testis. The cauda epididymis is full of spermatozoa and the accessory glands are in a high state of activity from October to the end of March. Evidently, the changes in these structures are synchronous with the activity of the testis.

Hipposideros fulvus fulvus

This species breeds once a year in a sharply defined season (Madhavan *et al.* 1978). Copulation followed by conception occurs in all adult females in the colony in the middle of November and each female delivers a single young one between 23 April and 7 May.

During the breeding season the colony contains some immature males with juvenile testes and accessory glands. This indicates that males do not attain sexual maturity in the year of birth although they reach adult body weight within 7-8 months of age. Since the condition of the male genitalia of all the juvenile specimens was similar, it is evident that they are all nearly of the same age. This also indicates that animals born during April-May attain sexual maturity by the following September and participate in copulation in November, when they are about 18-19 months old.

The testis weight of adults during the different months of the year is given in Fig. 3. The testis weight remains low until September when it suddenly increases, reaching its maximum in October, then falls to low values in February. During the sexually quiescent period the testis weight of adults falls below the testis weight of animals approaching adolescence.

Microscopic examination reveals that the testis of adult animals present a typical regressed picture from January to about the end of August. The seminiferous tubules are small in diameter with narrow lumina. The germinal epithelium is composed of a layer of resting spermatogonia which do not exhibit any division stages. A loose parenchymatous connective tissue with mostly fusiform cells and a few clusters of interstitial cells occur in the intertubular areas. The testis is quiescent until August when it suddenly spurts into spermatogenetic activity which occurs vigorously during September, October and November. During this period the seminiferous tubules increase in diameter and have all stages of spermatogenesis. The cauda epididymis is full of spermatozoa during September to December, when the accessory glands are also in a state of intense activity.

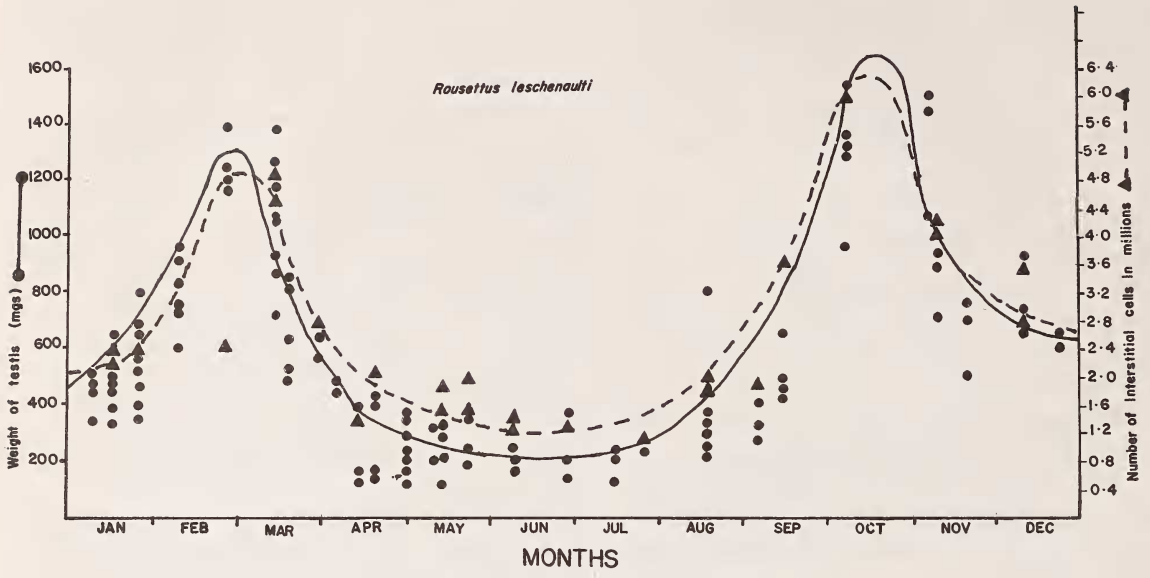


Fig. 2. Variations in weight of the adult testes and the number of interstitial cells during different months of the year in *Rousettus leschenaulti*. See text for descriptions.

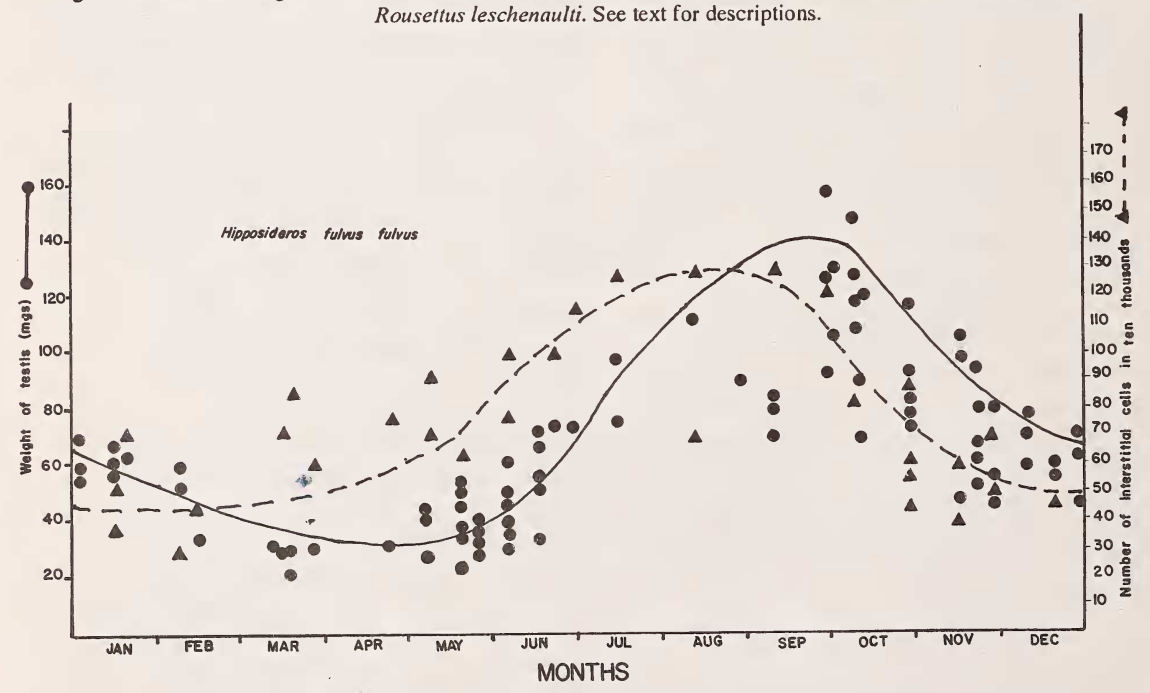


Fig. 3. Variations in weight of the adult testes and interstitial cells during different months of the year in *Hipposideros fulvus fulvus*. See text for descriptions.

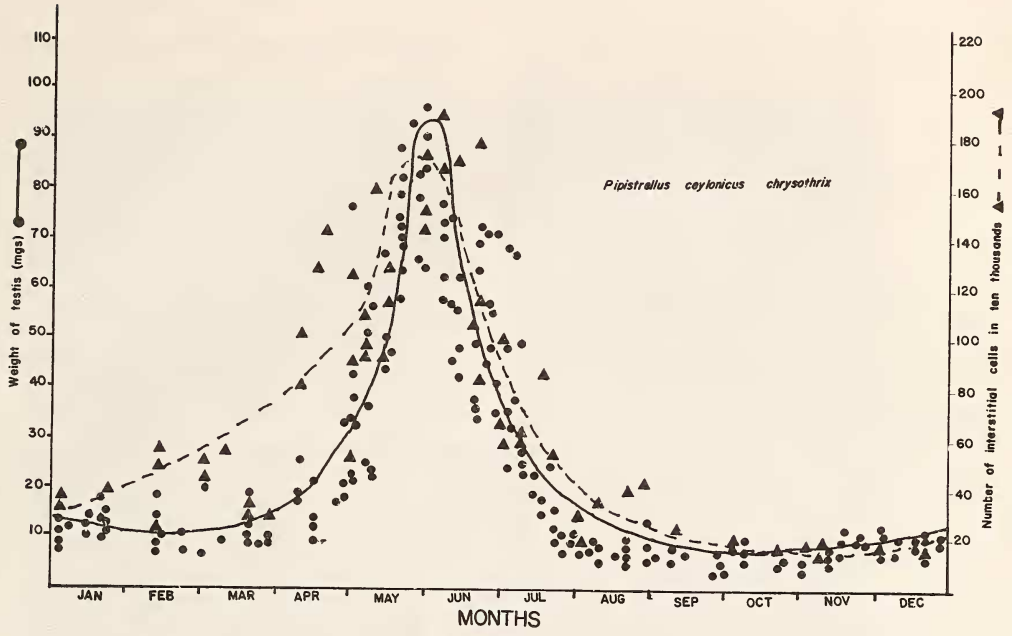


Fig. 4. Weight of adult testes and the number of interstitial cells during different months of the year in *Pipistrellus ceylonicus chrysothrix*. See text for descriptions.

The changes in the number of interstitial cells in the adult testis during the different months of the year are indicated in Fig. 3. The curve in the figure indicates that the interstitial cells are abundant during June to September, and become less numerous during the rest of the year. The period of highest abundance corresponds to the time just prior to commencement of spermatogenetic activity; the time when they start declining in number coincides with the period when the testis exhibits vigorous spermatogenetic activity. Evidently, regression of the interstitial cells in this species commences before the testis exhibits high spermatogenetic activity.

Pipistrellus ceylonicus chrysothrix

Copulation occurs during the first week of June, but ovulation does not occur until the second week of July (Madhavan 1971, Gopalakrishna and Madhavan 1979). During this period the inseminated spermatozoa are stored in the female genital tract (Gopalakrishna and Madhavan 1979). Each female delivers normally two, and rarely three young ones between the last week of August and the first half of September.

Males grow rapidly and attain sexual maturity when they are 8-9 months old. Hence, all males in the colony are sexually mature during the reproductive season.

Fig. 4 gives the testis weight of adult males during different months of the year. The testis weight commences to increase from April and attains the maximum during the second half of May, after which it progressively decreases and reaches low values from July to the end of the following March. Histological examination reveals that the testis is quiescent from July to the following March. Early spermatogenetic activity is noticed during April when the testis contains mostly spermatogonia and spermatocytes. Vigorous meiotic divisions and spermateleosis occur during May and continue (but less vigorously) during June. Hence, during this period the seminiferous tubules contain large

numbers of spermatozoa and the epididymides are full of spermatozoa. The accessory glands are in a high state of activity from the middle of May until the first week of July. This suggests that males are sexually active from May to the first half of July.

Discussion

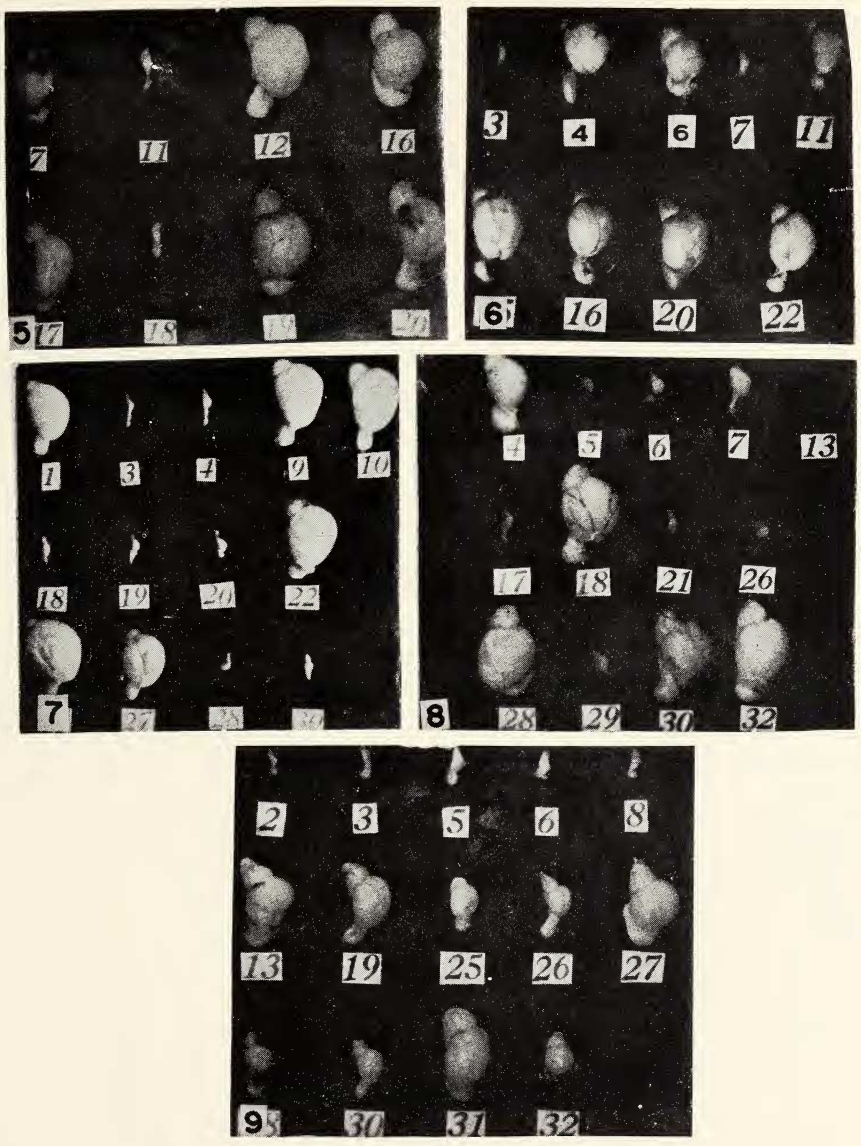
In *Rousettus leschenaulti,* where there are two strictly defined periods of heat in the females resulting in two litters in the year (Gopalakrishna and Choudhari 1977), the male seems to have a protracted period of elevated spermatogenetic activity and activity of accessory glands spanning both the cycles of the female. There is, however, a slight lowering of spermatogenetic activity in the testis and the secretory activity in the accessory glands during the period between the two periods of heat in the female, that is during December-February. It is, however, interesting to note that the regression in spermatogenetic activity in the testis and interstitial cells, and secretory activity in the accessory glands, does not fall to the level as in May when the animals are sexually quiescent. This suggests that elevated activity in the testis and accessory glands is maintained from October to the following April.

In *Hipposideros fulvus fulvus* there is only one sharply restricted breeding cycle in the year. However, while in the females oestrus is sudden and leads to copulation restricted to a strictly defined period in the latter half of November (Madhavan *et al.* 1979), spermatogenetic activity in the male extends for nearly three months — from September to November — and the epididymides are full of spermatozoa from September until December. The accessory glands are also most active from the third week of October to the middle of December. These facts suggest that while males are sexually competent from September through December, the period of copulation is determined by the female.

The situation in *Pipistrellus ceylonicus chrysothrix* is different from that in the other two species. Spermatogenetic and Leydig cell activity in the male commences in May and becomes most vigorous during the first half of June, after which it is considerably reduced until it ceases altogether by the second half of July. Accessory glands in the male are in the peak of activity during June and the first half of July. Thus, even after the abatement of spermatogenetic activity in the testis, the accessory glands remain active for a further period when the epididymides are full of spermatozoa. These facts suggest that the males retain their copulatory competence during June and the first half of July. This is probably an adaptation meant to serve those females which either missed copulation or were not successful during the early part of June, and to ensure that all females in the colony conceive. This is probably an adaptation to the far smaller number of males than females in the colony (Gopalakrishna and Madhavan 1970) and hence, each male needs to serve more than one female to ensure that all females conceive. In *P. c. chrysothrix* the spermatogenetic activity in the testis is not strictly synchronous with the activity of the accessory glands nor the activity in the female, unlike in the other two species in which there is a close synchrony of these activities. Females of this species have evidently developed a mechanism to store spermatozoa in the genital tract for several weeks prior to ovulation.

The fact that there are marked differences in the breeding rhythm of three species of bats inhabiting the same geographical region indicates that external factors such as temperature, rainfall and duration of daylight do not play a significant role in determining the breeding season of these bats.

H. f. fulvus and *P. c. chrysothrix* are approximately of the same size (8 to 10 g body weight) and roost in nearly identical kinds of places, and their food choice (small insects) is also the same. Yet their reproductive rhythms are different. Evidently, the reproductive peri-



Figs. 5 – 9. **Testes collected during different dates of the year. Note difference in sizes of testes** during each collection.
Fig. 5. Testes of specimens collected on 9 November 1965. Fig. 6. Testes of specimens collected on 16 January 1965.
Fig. 7. Testes of specimens collected on 9 February 1965. Fig. 8. Testes of specimens collected on 13 March 1965.
Fig. 9. Testes of specimens collected on 19 April 1965.
While during November to February the testes are of two distinct sizes, those collected in March and April are of three distinct sizes. See text for descriptions.